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(57) Abstract			
The present invention relates to sustained-release formulations using alginate gel beads and methods thereof.			
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SUSTAINED-RELEASE ALGINATE GELS

FIELD OF THE INVENTION

5 The present invention relates to sustained-release formulations using alginate gel beads and methods thereof.

BACKGROUND

10 With the advances in genetic and cell engineering technologies, the availability of recombinant proteins has engendered advances in the use of proteins as medicaments for therapeutic
15 applications. Many illnesses or conditions treated with pharmaceutical proteins require sustained protein levels to achieve the most effective therapeutic result. However, as with most protein pharmaceuticals, the generally short biological half-life requires
20 frequent administration. These repeated injections are given at various intervals which result in fluctuating medication levels at a significant physical and monetary burden on the patients. Since many conditions respond better to controlled levels of a
25 pharmaceutical, a need exists for controlled release of a medicament to provide longer periods of consistent release. Such sustained-release medicaments would provide the patient with not only enhanced prophylactic, therapeutic or diagnostic effects, but
30 also a decrease in the frequency of injections as well as in overall costs.

 Current attempts to sustain medication levels in humans or animals between doses have included the use of biodegradable polymers as matrices to control
35 medicament release. For example, Great Britain Patent No. 1,388,580 discloses the use of hydrogels for

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sustained-release of insulin. U.S. Patent No. 4,789,550 discloses the use of polylysine coated alginate microcapsules for delivery of protein by encapsulating living cells. Sustained-release attempts
5 have also utilized anionic or cationic polymer compositions surrounded by ionic polymers of the opposite charge for encapsulating cells capable of producing biologically active compositions. U.S. Patent No. 4,744,933. Likewise, multiple coats of anionic or
10 cationic cross-linking polymers have also been disclosed as means for obtaining controlled release. U.S. Patent Nos. 4,690,682 and 4,789,516. In addition, further attempts disclose the use of alginates alone, or alginates coated with other biodegradable polymers,
15 for controlled release of polypeptide compositions or cation precipitates thereof. PCT WO 96/00081, PCT WO 95/29664 and PCT WO 96/03116.

These attempts, however, have provided insufficient means for obtaining sustained-release
20 delivery of desired protein pharmaceuticals. It is generally known that the use of certain biodegradable polymers, e.g., polylactide co-glycolide, under *in vivo* conditions, exhibit high initial bursts of medicament release. Johnson, O. et al., Nature Med., 2/7: 795
25 (1996). Furthermore, it is generally known that proteins used with current forms of sustained-release preparations can undergo denaturation and lose their bioactivity upon exposure to the encapsulating agents. Such preparations use organic solvents which can have
30 deleterious effects on the protein of choice. Finally, as discussed below, use of alginate alone has not provided the desired controlled protein release necessary for effective therapeutic results.

In general, alginates are well known,
35 naturally occurring, anionic, polysaccharides comprised of 1,4-linked- β -D-mannuronic acid and α -L-guluronic

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acid. Smidsrod, O. et al., Trends in Biotechnology, 8: 71-78 (1990); Aslani, P. et al., J. Microencapsulation, 13/5: 601-614 (1996). Alginates typically vary from 70% mannuronic acid and 30% guluronic acid, to 30%
5 mannuronic acid and 70% guluronic acid. Smidsrod, supra. Alginic acid is water insoluble whereas salts formed with monovalent ions like sodium, potassium and ammonium are water soluble. McDowell, R.H., "Properties of Alginates" (London, Alginate Industries Ltd, 4th
10 edition 1977). Polyvalent cations are known to react with alginates and to spontaneously form gels.

Alginates have a wide variety of applications such as food additives, adhesives, pharmaceutical tablets and wound dressings. Alginates have also been
15 recommended for protein separation techniques. For example, Gray, C.J. et al., in Biotechnology and Bioengineering, 31: 607-612 (1988) entrapped insulin in zinc/calcium alginate gels for separation of insulin from other serum proteins.

20 Alginate matrices have also been well documented for drug delivery systems, see for example U.S. Patent No. 4,695,463 disclosing an alginate based chewing gum delivery system and pharmaceutical preparations. Alginate beads have been used for
25 controlled release of various proteins such as: tumor necrosis factor receptor in cation-alginate beads coated with polycations, Wee, S.F, Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 21: 730-31 (1994); transforming growth factor encapsulated in alginate
30 beads, Puolakkainen, P.A. et al., Gastroenterology, 107: 1319-1326 (1994); angiogenic factors entrapped in calcium-alginate beads, Downs, E.C. et al., J. of Cellular Physiology, 152: 422-429 (1992); albumin entrapped in chitosan-alginate microcapsules, Polk, A.
35 et al., J. Pharmaceutical Sciences, 83/2: 178-185 (1994), or chitosan-calcium alginate beads coated with

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polymers, Okhamafe, A. O. et al., J. Microencapsulation, 13/5: 497-508 (1996); hemoglobin encapsulated with chitosan-calcium alginate beads, Huguet, M.L. et al., J. Applied Polymer Science, 51: 1427-1432 (1994), Huguet, M.L. et al., Process Biochemistry, 31: 745-751 (1996); and interleukin-2 encapsulated in alginate-chitosan microspheres, Liu, L.S. et al., Proceed. Intern. Symp. Control. Rel. Bioact. Mater, 22: 542-543 (1995).

10 Systems using alginate gel beads, or alginate/calcium gel beads, to entrap proteins suffer from lack of any sustained-release effect due to rapid release of the protein from the alginate beads. Liu, L. et al., J. Control. Rel., 43: 65-74 (1997). To avoid
15 such rapid release, a number of the above systems attempt to use polycation polymer coatings (e.g., polylysine, chitosan) to retard the release of the protein alginate beads. See, e.g., Wheatley, M.A. et al., J. Applied Polymer Science, 43: 2123-2135 (1991);
20 Wee, S.F. et al. supra; Liu, L.S. et al. supra; Wee, S.F. et al., Controlled Release Society, 22: 566-567 (1995) and Lim, et al. supra.

 Polycations, such as polylysine, are positively charged polyelectrolytes which interact with
25 the negatively charged alginate molecules to form a polyelectrolyte complexes that act as diffusion barriers on the bead surface. Problems can occur with the use of polycations in that: (1) such formulations maybe cytotoxic due to the polycations (Huguet, M.L. et al., supra; Zimmermann, Ulrich, Electrophoresis, 13:
30 269 (1992); Bergmann, P. et al., Clinial Science, 67: 35 (1984)); (2) polycations are prone to oxidation; (3) beads with polycation coatings tend not to be erodible and build up in the body; (4) such formulations are
35 made via laborious coating procedures which include multiple coatings of the polycation polylysine (Padol,

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et al., Proceed. Intern. Symp. Control. Rel. Bioact. Mater, 2: 216 (1986) and (5) ionic interactions between the protein and the polycations can result in loss of protein activity or cause protein instability.

5 Accordingly, a need exists to develop pharmaceutical formulations which achieve a better means of sustained-release for clinical applications. Numerous recombinant or natural proteins could benefit from constant long term release and thereby provide
10 more effective clinical results.

 The present invention provides such advances. Pharmaceutical compositions of the present invention are capable of providing protein protection, decreased degradation and slow release with increased protein
15 stability and potency. Also, pharmaceutical compositions of the present invention provide a simple, rapid and inexpensive means of controlled recombinant protein release for effective prophylactic, therapeutic or diagnostic results.

20

SUMMARY OF THE INVENTION

 The present invention relates to sustained-release formulations using alginate gel beads or
25 particles, and methods thereof. In particular, the formation of the sustained-release gels includes the co-precipitation of alginate gel beads with a biologically active agent. This approach provides an advantage of producing efficient and high loading of
30 biologically active agent within the alginate gel for sustained-release delivery while achieving protein protection, decreased degradation, increased stability and potency of the agent to be delivered.

 Accordingly, one aspect of the present
35 invention provides a sustained-release composition, comprising a hydrophilic polymer; a biologically active

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agent; and at least one precipitating agent. During the formulation of the composition the biologically active agent is co-precipitated with the hydrophilic polymer. In addition, additional precipitating agents
5 may also be added to the composition. As used herein, the term co-precipitation refers to the use of agent(s) for precipitation of the biologically active agent together with the hydrophilic polymer so as to form a matrix of the precipitated polymer and agent, e.g.,
10 formation of alginate gel beads would be via precipitation. Such precipitation can be simultaneous or within close proximity thereto. The precipitation of molecules and any related precipitating agents are well known to those skilled in the art.

15 Another aspect provides for methods to produce the sustained-release compositions of the present invention. It comprises the steps of dissolving a biologically active agent and a hydrophilic polymer with a solvent to form a first
20 mixture; dissolving at least one precipitating agent in a solvent to form a second mixture; adding the biologically active agent and the hydrophilic polymer solution of the first mixture with the precipitating agent and solvent of the second mixture; and co-
25 precipitating the biologically active agent within the hydrophilic polymer. The present methods can also include the use of additional precipitating agents. In addition, a step for isolating the sustained-release composition is also contemplated.

30 As used herein, the term solvent refers to aqueous based solvents capable of dispersing or dissolving the biologically active agents, hydrophilic polymers or precipitating agents of choice. Such solvents are well known to one skilled in the art.
35 Addition of the first mixture with the second mixture to form the co-precipitation composition can be done by

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methods well known to one skilled in the art, including but not limited to droplet addition, dispersion, spraying or mixing by using spray jets, air jets, atomizing, and electric fields. The term dispersion for purposes of this invention can mean a liquid, solid or gaseous dispersions. As used herein, the term isolating, refers to the process for isolation of the sustained-release composition of the present invention. Such isolation and purification procedures are well known in the art.

In yet another aspect, the present invention provides for a sustained-release composition produced by the above methods. Further aspects include pharmaceutical formulations of the above compositions in a pharmaceutically acceptable carrier, or adjuvant.

In yet other aspects, the present invention provides for methods of treating indications with sustained-release compositions containing desired biologically active agents.

20

DETAILED DESCRIPTION OF THE INVENTION

Compositions

Hydrophilic polymers including alginates and derivatives thereof, can be obtained from various commercial, natural or synthetic sources well known in the art. As used herein, the term hydrophilic polymer refers to water soluble polymers or polymers having affinity for absorbing water. Hydrophilic polymers are well known to one skilled in the art. These include but are not limited to polyanions, including anionic polysaccharides such as alginate, carboxymethyl amylose, polyacrylic acid salts, polymethacrylic acid salts, ethylene maleic anhydride copolymer (half ester), carboxymethyl cellulose, dextran sulfate, heparin, carboxymethyl dextran, carboxy cellulose, 2,3-

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dicarboxycellulose, tricarboxycellulose, carboxy gum
arabic, carboxy carrageenan, carboxy pectin, carboxy
tragacanth gum, carboxy xanthan gum, pentosan
polysulfate, carboxy starch, carboxymethyl
5 chitin/chitosan, curdlan, inositol hexasulfate, β -
cyclodextrin sulfate, hyaluronic acid, chondroitin-6-
sulfate, dermatan sulfate, heparin sulfate,
carboxymethyl starch, carrageenan, polygalacturonate,
carboxy guar gum, polyphosphate, polyaldehyde-carbonic
10 acid, poly-1-hydroxy-1-sulfonate-propen-2,
copolystyrene maleic acid, agarose, mesoglycan,
sulfopropylated polyvinyl alcohols, cellulose sulfate,
protamine sulfate, phospho guar gum, polyglutamic acid,
polyaspartic acid, polyamino acids, derivatives or
15 combinations thereof. One skilled in the art will
appreciate other various hydrophilic polymers that are
within the scope of the present invention.

Likewise, precipitating agents can
be obtained from various commercial, natural or
20 synthetic sources which are well known in the art.
Precipitating agents include but are not limited to
polyvalent metal ions, salts, acetates, citrates,
chlorides, carbonates, hydroxides, oxalates, tartrates
or hydroxides thereof, acids or water soluble polymers.
25 In particular, the metal ions can include but are not
limited to aluminum, barium, calcium, iron, manganese
magnesium, strontium and zinc. Preferably the metal
ions are calcium and zinc or the salts thereof, like
zinc acetate, calcium acetate or chloride salts. Water
30 soluble small molecules and salts can also be used such
as ammonium sulfate, acetone, ethanol and glycerol.

As for water soluble polymers these include
but are not limited to polyethylene glycol, ethylene
glycol/propylene glycol copolymers,
35 carboxymethylcellulose, dextran, polyvinyl alcohol,
polyvinyl pyrrolidone, poly-1, 3-dioxolane,

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poly-1,3,6-trioxane, ethylene/maleic anhydride
copolymers, polyaminoacids, dextran, poly (n-vinyl
pyrrolidone) polyethylene glycol, propylene glycol
homopolymers, polypropylene oxide/ethylene oxide
5 copolymers, polyoxyethylated polyols, polyvinyl alcohol
succinate, glycerine, ethylene oxides, propylene
oxides, poloxamers, alkoxyated copolymers, water
soluble polyanions, derivatives or combinations
thereof. The water soluble polymer may be of any
10 molecular weight, and may be branched or unbranched.
For example, the preferred molecular weight of
polyethylene glycol is between about 700 Da and about
100 kDa for ease in handling and efficiency of
precipitation.

15 Other sizes and types of precipitating
agents, may be used, depending on the desired
therapeutic profile (e.g., the duration of sustained-
release desired, the effects, if any on biological
activity, the ease in handling, the degree or lack of
20 antigenicity and other known effects of a desired
precipitating agent to a therapeutic protein or
analog). One skilled in the art will appreciate other
precipitating agents that are within the scope of the
invention.

25 As used herein, the term buffer or buffer
solution refers to use of inorganic or organic acids or
a combination thereof to prepare a buffer solution as
known in the art. Inorganic acids within the scope of
the present invention include hydrogen halide (e.g.,
30 hydrochloric acid), phosphoric, nitric or sulfuric.
Other inorganic acids would be well known to one
skilled in the art and are contemplated herein. Organic
acids within the scope of the invention include
aliphatic carboxylic acids and aromatic acids such as
35 formic, carbonic, acetic, propionic, butyric, valeric,
caproic, acrylic, malonic, succinic, glutaric, adipic,

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maleic, fumaric, glycine or phenol sulfonic. Other organic acids would be well known to one skilled in the art. The preferred buffer of the present invention includes glycine and glycine phosphoric acid buffer systems.

As used herein, biologically active agents refers to recombinant or naturally occurring proteins, whether human or animal, useful for prophylactic, therapeutic or diagnostic application. The biologically active agent can be natural, synthetic, semi-synthetic or derivatives thereof. The biologically active agents of the present invention must be precipitable. A wide range of biologically active agents are contemplated. These include but are not limited to hormones, cytokines, hematopoietic factors, growth factors, antiobesity factors, trophic factors, anti-inflammatory factors, and enzymes (see also U.S. Patent No. 4,695,463 for additional examples of useful biologically active agents). One skilled in the art will readily be able to adapt a desired biologically active agent to the compositions of present invention.

Such proteins would include but are not limited to interferons (see, U.S. Patent Nos. 5,372,808, 5,541,293 4,897,471, and 4,695,623 hereby incorporated by reference including drawings), interleukins (see, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (see, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 5,621,080 hereby incorporated by reference including drawings), granulocyte-colony stimulating factors (see, U.S. Patent Nos. 4,810,643, 4,999,291, 5,581,476, 5,582,823, and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505 and 95/17206, hereby incorporated by reference including drawings),

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and the OB protein (see PCT publication Nos. 96/40912, 96/05309, 97/00128, 97/01010 and 97/06816 hereby incorporated by reference including figures). In addition, biologically active agents can also include but are not limited to anti-obesity related products, insulin, gastrin, prolactin, adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), human chorionic gonadotropin (HCG), motilin, interferons (alpha, beta, gamma), interleukins (IL-1 to IL-12), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), insulin-like growth factors (IGFs), macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), megakeratinocyte derived growth factor (MGDF), thrombopoietin, platelet-derived growth factor (PDGF), colony stimulating growth factors (CSFs), bone morphogenetic protein (BMP), superoxide dismutase (SOD), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein. The term proteins, as used herein, includes peptides, polypeptides, consensus molecules, analogs, derivatives or combinations thereof.

Derivatives of biologically active agents may included the attachment of one or more chemical moieties to the protein moiety. Chemical modification of biologically active agents has been found to provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. One skilled in the art will be able to select the desired chemical modification based on the desired dosage,

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circulation time, resistance to proteolysis, therapeutic uses and other considerations.

Complexes

5 The proteins, analog or derivative may be administered complexed to a binding composition. Such binding composition may have the effect of prolonging the circulation time of the protein, analog or derivative or enhancing the activity of the

10 biologically active agent. Such composition may be a protein (or synonymously, peptide), derivative, analog or combination. For example, a binding protein for the OB protein is OB protein receptor or portion thereof, such as a soluble portion thereof. Other binding

15 proteins may be ascertained by examining OB protein, or the protein of choice, in serum, or be empirically screening for the presence of binding. Such binding will typically not interfere with the ability of OB protein or analog or derivative to bind to endogenous

20 OB protein receptor and/or effect signal transduction. In addition to the OB protein, binding complexes will also be applicable to other therapeutic proteins of the present invention as well. Those well skilled in the art will be able to ascertain appropriate binding

25 proteins for use with the present invention.

Pharmaceutical Compositions

 The sustained-release pharmaceutical compositions of the present invention may be

30 administered by oral (e.g., capsules such as hard capsules and soft capsules, solid preparations such as granules, tablets, pills, troches or lozenges, cachets, pellets, powder and lyophilized forms, liquid preparations such as suspensions) and non-oral

35 preparations (e.g., intramuscular, subcutaneous, transdermal, visceral, IV (intravenous), IP

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(intraperitoneal), intraarterial, intrathecal, intracapsular, intraorbital, injectable, pulmonary, nasal, rectal, and uterine-transmucosal preparations).

In general, comprehended by the invention are
5 sustained-release pharmaceutical compositions comprising effective amounts of protein, or derivative products, with the sustained-release compositions of the invention together with pharmaceutically acceptable
10 diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers needed for administration. See PCT 97/01331 hereby incorporated by reference. The optimal pharmaceutical formulation for a desired biologically active agent will be determined by one
15 skilled in the art depending upon the route of administration and desired dosage. Exemplary pharmaceutical compositions are disclosed in Remington's Pharmaceutical Sciences (Mack Publishing Co., 18th Ed., Easton, PA, pgs. 1435-1712 (1990)).

Components that may be needed for
20 administration include diluents of various buffer content (e.g., Tris-HCl, acetate), pH and ionic strength; additives such as surfactants and solubilizing agents (e.g., Tween 80, HCO-60, Polysorbate 80), anti-oxidants (e.g., ascorbic acid,
25 glutathione, sodium metabisulfite), additional polysaccharides (e.g., carboxymethylcellulose, sodium alginate, sodium hyaluronate, protamine sulfate, polyethylene glycol), preservatives (e.g., Thimersol, benzyl alcohol, methyl paraben, propyl paraben) and
30 building substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic/polyglycolic acid polymers or copolymers, etc. or combined with liposomes. Hylauronic acid may
35 also be used as an administration component and this may have the effect of promoting even further the

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sustained duration in the circulation. Additionally, sustained-release compositions of the present invention may also be dispersed with oils (e.g., sesame oil, corn oil, vegetable), or a mixture thereof with a phospholipid (e.g., lecitin), or medium chain fatty acid triglycerides (e.g., Miglyol 812) to provide an oily suspension. The compositions of the present invention may also be dispersed with dispersing agents such as water-soluble polysaccharides (e.g., mannitol, lactose, glucose, starches), hyaluronic acid, glycine, fibrin, collagen and inorganic salts (e.g., sodium chloride).

In addition, also contemplated for use in the administration of the sustained-release compositions of the present invention are mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

The administration components may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. One skilled in the art will appreciate the appropriate administration components and/or the appropriate mechanical devices to use depending on the therapeutic use, route of administration, desired dosage, circulation time, resistance to proteolysis, protein stability and other considerations.

30

Methods of Use

Therapeutic. Therapeutic uses depend on the biologically active agent used. One skilled in the art will readily be able to adapt a desired biologically active agent to the present invention for its intended

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therapeutic uses. Therapeutic uses for such agents are set forth in greater detail in the following publications hereby incorporated by reference including drawings. Therapeutic uses include but are not limited to uses for proteins like interferons (see, U.S. Patent Nos. 5,372,808, 5,541,293 4,897,471, and 4,695,623 hereby incorporated by reference including drawings), interleukins (see, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (see, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 5,621,080 hereby incorporated by reference including drawings), granulocyte-colony stimulating factors (see, U.S. Patent Nos. 4,999,291, 5,581,476, 5,582,823, 4,810,643 and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505 and 95/17206, hereby incorporated by reference including drawings), and the OB protein (see PCT publication Nos. 96/40912, 96/05309, 97/00128, 97/01010 and 97/06816 hereby incorporated by reference including figures).

In addition, therapeutic uses of the present invention include uses of biologically active agents including but not limited to anti-obesity related products, insulin, gastrin, prolactin, adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), human chorionic gonadotropin (HCG), motilin, interferons (alpha, beta, gamma), interleukins (IL-1 to IL-12), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), insulin-like growth factors

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(IGFs), macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), thrombopoietin, platelet-derived growth factor (PDGF), colony stimulating growth factors (CSFs), bone morphogenetic protein (BMP), superoxide dismutase (SOD), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein. The term proteins, as used herein, includes peptides, polypeptides, consensus molecules, analogs, derivatives or combinations thereof. In addition, the present compositions may also be used for manufacture of one or more medicaments for treatment or amelioration of the conditions the biologically active agent is intended to treat.

By way of example, therapeutic uses (oxygenation in the blood) and a decrease in bone resorption or osteoporosis may also be achieved in the absence of weight loss.

Combination Therapies. The present compositions and methods may be used in conjunction with other therapies, such as altered diet and exercise. Other medicaments, such as those useful for the treatment of diabetes (e.g., insulin, and possibly amylin), cholesterol and blood pressure lowering medicaments (such as those which reduce blood lipid levels or other cardiovascular medicaments), activity increasing medicaments (e.g., amphetamines), diuretics (for liquid elimination), and appetite suppressants. Such administration may be simultaneous or may be in seriatim. In addition, the present methods may be used in conjunction with surgical procedures, such as cosmetic surgeries designed to alter the overall appearance of a body (e.g., liposuction or laser surgeries designed to reduce body mass, or implant surgeries designed to increase the appearance of body mass). The health benefits of cardiac surgeries, such

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as bypass surgeries or other surgeries designed to relieve a deleterious condition caused by blockage of blood vessels by fatty deposits, such as arterial plaque, may be increased with concomitant use of the present compositions and methods. Methods to eliminate gall stones, such as ultrasonic or laser methods, may also be used either prior to, during or after a course of the present therapeutic methods. Furthermore, the present methods may be used as an adjunct to surgeries or therapies for broken bones, damaged muscle, or other therapies which would be improved by an increase in lean tissue mass.

Dosages

One skilled in the art will be able to ascertain effective dosages by administration and observing the desired therapeutic effect. The dosage of the sustained-release preparation is the amount necessary to achieve the effective concentration of the biologically active agent in vivo, for a given period of time. The dosage and the preferred administration frequency of the sustained-release preparations varies with the type of the biologically active agent, the desired duration of the release, the target disease, desired administration frequency, the subject animal species and other factors. Preferable, the formulation of the molecule will be such that between about 0.10 ug/kg/day and 100 mg/kg/day will yield the desired therapeutic effect.

The effective dosages may be determined using diagnostic tools over time. By way of example, the present invention provides the dosages of OB protein. For example, a diagnostic for measuring the amount of OB protein in the blood (or plasma or serum) may first be used to determine endogenous levels of OB protein. Such diagnostic tool may be in the form of an antibody

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assay, such as an antibody sandwich assay. The amount of endogenous OB protein is quantified initially, and a baseline is determined. The therapeutic dosages are determined as the quantification of endogenous and
5 exogenous OB protein (that is, protein, analog or derivative found within the body, either self-produced or administered) is continued over the course of therapy. For example, a relatively high dosage may be needed initially, until therapeutic benefit is seen,
10 and then lower dosages used to maintain the therapeutic benefits.

Methods of Preparation

Protein/Alginate Bead Preparation. A typical
15 procedure is illustrated by the following example using the OB protein or leptin as the protein of choice. One skilled in the art will understand and be able to apply these procedures to other biologically active agents.

Preparation of the Drop Mixture. The term
20 "drop mixture" as used herein refers to the mixture containing the hydrophilic polymer and the biologically active agent. A one mL mixture of 5% alginate (10 mM TRIS; pH 8) is added with magnetic stirring to 4 mL Leptin (100 mg/mL; 10mM TRIS; pH 8) in a 10 mL beaker
25 (in an ice bath). The mixture becomes cloudy. Then 40 mL of 4 mM NaOH is added to the mixture and stirring is continued for 15 minutes (on ice). The mixture clarifies and its final pH is between approximately 8.6 to 8.8. The alginate concentration
30 should at least be 0.05% by weight. In addition, the alginate should preferably be at least 30% guluronic acid.

In addition to the above, polyethylene glycol can be added to the drop mixture as discussed below.
35 Likewise, buffers or excipients are helpful with stability of the protein of choice. One skilled in the

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art will be well aware of the appropriate ingredients that should be added for stability purposes depending on the protein chosen for delivery.

Preparation Of The Bath Mixture. The term
5 "bath mixture" as used herein refers to the mixture which contains the precipitating agent(s) used for co-precipitation of the biologically active agent and the hydrophilic polymer. The bath typically contains 10 mL of mixture, in a 50 mL beaker consisting of 100 mM CaCl₂
10 plus other ingredients (see below). The pH is preferably acidic to help decrease the burst effect. The pH should be preferably less than pH 4. The buffer in the bath will also depend on the protein used. One skilled in the art will be able to adjust the buffer
15 capacity or strength based on the protein used. Thus depending of the stability of the protein, if the buffer concentration is too high, for example with G-CSF, the protein may appear to be less stable and it will diminish the sustained-release.

20 The bath can be comprised of CaCl₂, ZnCl₂, polyethylene glycols ("PEG") and acidic buffers. The zinc interacts with the protein in precipitating it thereby helping to increase loading of the bead, decrease the burst effect and slow release of the
25 protein from the bead. The calcium helps to form the alginate precipitate and formation of the bead. Calcium also helps to shape the bead, especially if the bath is viscous because of the addition of other additives like PEG. Calcium can be increased when you have increase
30 viscosity to help maintain bead shape. Zinc concentrations should be at least 0.1 mM and calcium concentration should at least be 10mM.

The addition of PEG helps to increase the loading. Certain PEGs are known to precipitate
35 proteins. PEG can also be added to the protein/alginate mixture that is dropped into the bath

- 20 -

to help maximize the loading and sustained-release. The molecular weight of PEG can range from 700 Da to 1000 kDa, but preferably 700 Da - 100 kDa. One skilled in the art will be aware of the amount of PEG to add to the bath mixture but it can be as high as 99%, preferable less than 75% by weight. One skilled in the art will also be aware that the PEG concentration can be limited by viscosity of the bath.

10 Bead Preparation.

In general, droplets of a leptin/alginate drop mixture are sprayed, dripped or dispersed into a bath mixture (as described above) that precipitate or gels the leptin/alginate mixture. In addition, electrostatic means can be used for bead formation.

15 To make small beads, i.e. less than a few hundred microns in diameter, a flow chamber (nozzle holder) consisting of a needle with coaxial air flow is used. One of two ports is connected to a gas line and the other port to a syringe (3 mL) used to pump (at approximately 1 mL/min) the protein/alginate mixture into the bath. Typically 2 mL of the mixture is injected into 10 mL of bath mixture. The nozzle is positioned approximately 0.8 cm from the top of the bath beaker. The bead size is primarily determined by the gas flow rate, e.g., at a flow rate of 8 L/min the bead size ranges from 50-150 micron in diameter. The leptin/alginate flow rate has a much lesser effect on the bead size.

25 To make large (i.e., 1-3 mm diameter) beads, a 1 cc tuberculin syringe, fitted with a 24G needle is used to drip the leptin/alginate mixture into the bath mixture. The bath typically contains 1.5% CaCl₂ and 5 to 50 mM ZnCl₂. The beads are collected by pouring them through a 40 micron nylon cell strainer. The beads are rinsed on the strainer with 5 mL sterile

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water and gently blotted from the underside of the strainer with a cleanroom wiper (gamma Wipe 67). The beads are stored in a sterile plastic screw cap microtube.

5

Bead Loading

Burst Method: The drug loading of a selected group of beads is determined by accurately weighing 100 mg of the hydrated loaded beads into 1 mL of 0.5M sodium citrate pH 8.5. The bead suspension is incubated at room temperature until the beads disintegrate usually forming a precipitate. The suspension is centrifuged at 14K rpm for 2 min. (eppendorf, 5415 C). The supernatant is collected and absorbance at 280 nm is recorded. The precipitate is dissolved by suspending it in 1 mL of 7M urea. The absorbance of this mixture is recorded. The protein loading of the hydrated loaded beads is expressed as mg protein per mg bead or mg protein per mL bead and determined from the sum of the two absorbances.

15

Cumulative Method: This method is used in conjunction with the in vitro release studies. The amount of protein released from the beads including the burst at the end of the study is totalled. For details, see below.

20

In Vitro Release Studies

Hydrated loaded beads (100 mg) are weighed into a 1.5 mL microcentrifuge tube (eppendorf) and 1 mL buffer (10 mM histidine pH 7.4) added. The sample is placed in an incubator shaker at 37°C and 100-200 rpm. At selected time intervals, the sample is removed from the incubator, centrifuged (eppendorf, 1000 rpm, 2 min) and the supernatant is removed and replaced with 1 mL of fresh buffer. The amount of protein released is

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determined from the absorbance of the supernatant. After the final released sample has been taken the amount left in the bead is determined by the Bead Loading/Burst Method. The percent released at a given
5 time is determined from the summation of the total protein released and that remaining in the bead at the completion of the experiment.

In Vivo Studies

10 Mouse Weight Loss: In general, mice are injected once with a suspension of the loaded beads or unloaded beads. Six to eight week old female mice are used (type C57/BLC), typically weighing 20 grams. In the case of bead samples, 350 mcL of buffer (50 mM MES
15 pH6.7) is added to 100 mg of hydrated beads and vortexed. The suspension is drawn up into a 1cc syringe and all the beads and 300 mcL of the buffer are injected (23G needle) subcutaneously into the neck of the mouse. The mice are weighed daily.

20 Rat Pharmacokinetic Study: Six to eight week old female rats are used (type Sprague Dawley), typically weighing 250 grams. The injections are performed in a similar manner to that described in the
25 mouse weight loss experiments. Blood is sampled by catheter collection at various time intervals post injection and the samples analyzed for leptin by an ELISA assay.

30 EXAMPLES

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof. In addition,
35 with respects to the above disclosure or the examples

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below, one skilled in the art will be able to make the necessary changes to the disclosures for large scale production.

5 Example 1

 This example examines the effect of leptin concentration in the bead on the release of leptin from zinc/leptin coprecipitated alginate beads. The small beads are prepared as described above using 25 mM ZnCl₂ in the bath. The higher concentration bead, i.e. 66 mg/mL leptin, is prepared using 84 mg/mL leptin in 1% alginate while the lower concentration bead, i.e. 21 mg/mL leptin is prepared from 28 mg/mL leptin in 1% alginate. As the concentration of the leptin in the bead increases the fractional release of leptin from the bead decreases. For the higher concentration leptin 25% is released at 80h, while at the lower concentration 80% is released at 80h.

20 Example 2

 This example examines the effect of bath ZnCl₂ level on the release of leptin from zinc/leptin coprecipitated alginate beads. The small beads are prepared as described above but the ZnCl₂ level in the bath is at 0.5 and 25 mM and the concentration of leptin in the beads is 37 mg/mL (by cumulative method). This example shows that as the ZnCl₂ level in the bath increases the resultant beads have a decreased burst and decreased release rate of leptin. At 0.5 mM ZnCl₂, the beads have a 20% burst and 50% release at 40h; while at 25mM ZnCl₂ the beads have less than 5% burst and 25% release at 40h.

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Example 3

This example compares a zinc/leptin coprecipitate alginate bead with a control acetate buffer formulation (20 mg/mL) in a combined pharmacokinetic/bioactivity experiment. The small beads contain 64 mg/mL leptin (i.e., per mL of beads) and are fabricated as described above with 17 mM ZnCl₂ in the bath. Female rats (220g body weight) are given a single SC (subcutaneous) injection at 50 mg/kg dose. The plasma concentrations of the bead sample is sustained relative to that of the control. The rats injected with bead samples maintain a plasma concentration of leptin of over 50 ng/mL for over 112 h in contrast to that of 12-18 h for the control animals. The higher more sustained leptin blood levels in the bead group correlated with its more pronounced and sustained weight loss compared to the control group. The rats injected with bead samples continually lose weight for 120h; at 120h the total weight loss is 9% of the initial weight. In contrast, the control rats lose 7% of their initial weight in 50h but regain the weight by 120h.

Example 4

This example shows the effect of various PEGs in the bath, in addition to 10 mM ZnCl₂, on the efficiency of loading and in vitro release of IL-1ra. The small beads are prepared as described above with IL-1ra in 10 mM PIPES pH 6.85. One bead bath (A) contains 100mM CaCl₂, 10 mM ZnCl₂, 20% 1K PEG and 20% 2K PEG. A second bead bath (B) contains the same as A but without 20 % 1K PEG. The concentration of IL-1ra in beads A and B is 58 mg/mL, i.e. a loading efficiency of 74% as determined from the sodium citrate burst. The B formulation has a 55% burst and 75% released after 18h. The A formulation has a 20% burst and 50%

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released after 18h. Thus the addition of PEGs in the bath lead to highly loaded beads that sustain the release on the protein. Also, the addition of 1K PEG leads to an even lower burst and slower release of protein.

Example 5

This example shows the effect of having PEGs, but no zinc, in the bath on the loading of and initial burst of IL-1ra from alginate beads. The small beads are prepared as described above except the bath contains 20% 1K and 20% 2K PEG in addition to 100 mM CaCl₂. The efficiency of loading is 93% with 63 mg/mL IL-1ra in the bead. The initial burst is 35%. Thus, the addition of PEGs to the bath can lead to high protein loading without the presence of zinc ions.

Example 6

In this example a comparison is made of effectiveness of the release of a bolus injection of IL-1ra in buffer (10 mM PIPES, pH6.85) and IL-1ra in alginate beads of Example 1. Female Balb/C mice (20g body weight) are injected SC (subcutaneously) at time zero with the various formulations each containing 10 mg of IL-1ra. At 18h the mice are injected IV (intravenously) with rhIL-1B (0.1 mcg per mouse) and then killed 2h thereafter for blood sampling. Blood is analyzed for glucose concentration and lymphocyte numbers. IL-1beta normally causes a drop in glucose concentration and lymphocyte numbers but the presence of a certain level of IL-1ra protects against such a loss. The result of the experiment shows that only the mice that receive the IL-1ra contained in the beads are protected against the loss in value of the blood

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parameters. This results demonstrates that the alginate beads sustain the release of the IL-1ra at an effective level for at least 18h.

5 Example 7

 This example is a control experiment illustrating the preparation and release of protein containing beads using GCSF where the precipitation bath only contains CaCl₂ (100 mM). The large beads are prepared as described above. The syringe mixture contains 46 mg/mL GCSF (10 mM TRIS pH7) in 1% alginate. The prepared beads contain 16 mg/mL GCSF (from citrate burst). Thus with only CaCl₂ in the bath the efficiency of loading is 35%. The fractional release of the protein shows a 60% burst and 75% release in one day. Thus using a known procedure described in the literature one obtains low protein loading and rapid release.

20 Example 8

 This example shows the effect of ZnCl₂ in the bath on the loading and release of GCSF in alginate beads. The large beads are prepared as described above except 10 mM ZnCl₂ is added to the bath. The syringe mixture contains 46 mg/mL GCSF in 1% alginate. The prepared beads contain 28 mg/mL GCSF (from citrate burst). Thus with the addition of 10 mM ZnCl₂ to the bath (in addition to 100 mM CaCl₂) the efficiency of loading increases from 35% (Example 6) to 61%. The fractional release of the protein shows a reduced burst of 40% and a one day release of 55%. Thus the addition of ZnCl₂ to the CaCl₂ bath leads to a higher loading efficiency, lower burst and slower release of protein.

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Example 9

This example shows the effect of having PEG in the bath with the bath pH being acidic on the loading and release of GCSF with alginate beads. The large beads are prepared as described in Example 7 except 20% PEG (Aldrich) is added to the bath and the bath pH is 1.7. The bath also contains 100 mM CaCl₂ and 10 mM ZnCl₂. The loading efficiency for GCSF is 54% and the fractional release (25 mg/mL in the beads) shows a much reduced burst of less than 5% and 40% release after 100 hours. Thus an acidic bath mixture that can contain PEG (in addition to CaCl₂ and ZnCl₂) leads to low burst and slow release of protein.

Example 10

This example shows the effect of having PEGs and zinc in the bath and the bath pH lowered with acidifying agent(s) on the loading of and the initial burst of GCSF from alginate beads. The large beads are prepared as described above except the bath contains 25 mM ZnCl₂, 100 mM CaCl₂, and 5% PEG 1K and 5% PEG 10K. The bath pH is lowered with glycine buffer and phosphoric acid to pH 1.65. The resultant beads (20 mg/mL loading) exhibit less than 5% burst and a fractional release of 40% in 90h. Thus a combination of PEGs and zinc and low pH in the bath leads to a low burst and slow release.

Example 11

This example shows the preparation of GCSF in alginate beads with PEGs in a low pH bath without the addition of zinc ions. The small beads are prepared as described above except the bath contains 5% 1K and 5% 10K PEG. The bath pH is lowered to 1.43 using glycine and phosphoric acid buffer. The efficiency of loading is 42% with 14 mg/mL in the beads (from citrate burst).

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The fractional release shows a 32% burst and 35% release after 70h.

Example 12

5 The large GCSF/ alginate beads of Example 12, and Examples 13-15 below, are prepared in a similar manner to that described above but with more stringent control of the timing of the various operations and an alternate method to determine loading. More
10 specifically, 1 mL of a GCSF/alginate mixture is dripped into 10 mL of the magnetically stirred bath in approximately 2 minutes. The beads are filtered and washed with 5 mL of water. The total bead making procedure takes approximately 5 minutes. The loading
15 is determined (by A280) from the difference in the amount of protein in the alginate mixture dripped into the bath and the protein that does not get incorporated into the formed beads, i.e. the protein remaining in the bath mixture and the washes. This amount of
20 protein incorporated into the beads is divided by the 1 mL volume of mixture added to the bath to obtain the loading expressed in mg/mL of beads.

Example 12 compares the presence of PEG in the bath on the loading of GCSF into the beads. The
25 large beads are prepared as described above except the bath contains 200 mM CaCl₂ and 15% PEG 8K (pH 5-6); the bath of the control beads has 200 mM CaCl₂. The addition of PEG to the bath increases the loading from 21.8 mg/mL (70% efficiency) to 26.5 mg/mL (85%
30 efficiency).

Example 13

This example shows the effect of low bath pH on the loading and release of GCSF with alginate beads.
35 The bead formation and loading are determined as in Example 12 with PEG except one of the baths contains

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0.5 M glycine buffer pH 2.1. The loading at pH 2.1, 24.9 mg/mL (80% efficiency), is similar to that of the control. However, the initial release at one hour (29%) is lower and the 24 h release more sustained (32%)
5 than that of the control (92% and 99% respectively).

Example 14

This example shows the effect of the addition of zinc to a bath containing PEG on the loading of GCSF
10 into alginate beads. The bead formation and loading are determined as in Example 12 with PEG. The addition of 10 mM ZnCl₂ to the bath increased the loading from 26.5 mg/mL (85% efficiency) to 30.3 mg/mL (97% efficiency).

15 Example 15

This example shows a low initial burst and sustained-release of GCSF from alginate beads. The bead formation, loading and release are performed in a similar manner to that in EXAMPLE 13 except the bath
20 contains 100 mM CaCl₂, 5% PEG 1K and 5% PEG 2K, and 0.5 M glycine buffer (pH 2.1). The loaded beads contain 24 mg/mL GCSF. At 1/2 h the initial release is near zero, at 19 h the release is 13.6% and at 44 h the release is 24%.

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CLAIMS

We Claim:

- 5 1. A sustained-release composition,
 comprising:
 - a) a hydrophilic polymer;
 - b) a biologically active agent; and
 - c) at least one precipitating agent;
- 10 characterized in that the biologically active agent is
 co-precipitated within the hydrophilic polymer.
2. The composition of Claim 1 wherein the
 precipitating agent is selected from the group
- 15 consisting of polyvalent metal ions or salts, acetates,
 citrates, chlorides, carbonates or hydroxides thereof.
3. The composition of Claim 2 wherein the
 metal ion is selected from the group consisting of
- 20 manganese, strontium, iron, magnesium, calcium, barium,
 aluminium or zinc.
4. The composition of Claim 3 wherein the
 precipitating agent is a polyvalent ion selected from
- 25 the group consisting of zinc, calcium or a combination
 thereof.
5. The composition of Claim 1 wherein the
 hydrophilic polymer is a polyanion.
- 30 6. The composition of Claim 1 wherein the
 hydrophilic polymer is a polysaccharide.
7. The composition of Claim 6 wherein the
- 35 polysaccharide is an acidic polysaccharide.

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8. The composition of Claim 7 wherein the polysaccharide is alginate.

5 9. The composition of Claim 8 wherein the alginate contains at least 30% guluronic acid.

10 10. The composition of Claim 8 wherein the alginate consist of at least 0.05% by weight.

11. The composition of Claim 1 wherein the biologically active agent comprises a protein.

15 12. The composition of Claim 11 wherein the protein consist of at least 0.01 mg/ml.

20 13. The composition of Claim 11 wherein the protein is selected from the group consisting of hematopoietic factors, colony stimulating factors, anti-obesity factors, growth factors, trophic factors, and antiinflammatory factors.

25 14. The composition of Claim 11 wherein the protein is selected from the group consisting of leptin, G-CSF, SCF, BDNF, GDNF, NT3, GM-CSF, IL-1ra, IL2, TNF-bp, MGDF, OPG, interferons, erythropoietin, KGF and analogs or derivatives thereof.

30 15. The composition of Claim 1 further comprising at least two precipitating agents.

35 16. The composition of Claim 15 wherein at least one of the precipitating agents is selected from the group consisting of water soluble polymers.

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17. The composition of Claim 16 wherein the water soluble polymer is polyethylene glycol.

18. A method of producing a sustained-
5 release composition, comprising the steps of:
a) dissolving a biologically active agent and a hydrophilic polymer with a solvent to form a first mixture;
b) dissolving at least one
10 precipitating agent in a solvent to form a second mixture;
c) adding the first mixture with the second mixture; and
d) co-precipitating the biologically
15 active agent with the hydrophilic polymer to form a co-precipitated particle.

19. The method of Claim 18 wherein the precipitating agent is selected from the group
20 consisting of polyvalent metal ions or salts, acetates, citrates, chlorides, carbonates or hydroxides thereof.

20. The composition of Claim 19 wherein the metal ion is selected from the group consisting of
25 manganese, strontium, iron, magnesium, calcium, barium, aluminium or zinc.

21. The method of Claim 20 wherein the precipitating agent is a polyvalent ion selected from
30 the group consisting of zinc, calcium or a combination thereof.

22. The method of Claim 21 wherein the precipitating agent in the second mixture consists of
35 at least 1 mM of calcium and 0.1 mM of zinc.

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23. The method of Claim 18 wherein the hydrophilic polymer is a polyanion.

5 24. The method of Claim 18 wherein the hydrophilic polymer is a polysaccharide.

 25. The method of Claim 24 wherein the polysaccharide is an acidic polysaccharide.

10 26. The method of Claim 25 wherein the polysaccharide is alginate.

 27. The method of Claim 26 wherein the
15 alginate contains at least 30% guluronic acid.

 28. The method of Claim 27 wherein the first mixture consists of at least 0.05% alginate by weight.

20 29. The method of Claim 18 wherein the biologically active agent comprises a protein.

 30. The method of Claim 29 wherein the first mixture consists of at least 0.01 mg/ml protein.

25 31. The method of Claim 29 wherein the protein is selected from the group consisting of hematopoietic factors, colony stimulating factors, anti-obesity factors, growth factors, trophic factors,
30 and antiinflammatory factors.

 32. The method of Claim 29 wherein protein is selected from the group consisting of leptin, G-CSF, SCF, BDNF, OPG, GDNF, NT3, GM-CSF, IL-1ra, IL2, TNF-bp,
35 MGDF, interferons, erythropoietin, KGF and analogs or derivatives thereof.

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33. The method of Claim 18 further comprising at least two precipitating agents in the second mixture.

5

34. The method of Claim 33 wherein at least one of the precipitating agents is selected from the group consisting of water soluble polymers.

10

35. The method of Claim 34 wherein the water soluble polymer is polyethylene glycol.

15

36. The method of Claim 18 wherein adding the first mixture to second mixture occurs by spraying, electrostatic fields, droplet addition, dispersion, or mixing, to form the coprecipitated particles.

20

37. The method of Claim 18 further comprising the step of isolating the co-precipitated particle.

25

38. The sustained-release product produced by the method of Claims 18, 36 and 37.

39. A pharmaceutical formulation according to Claims 1 or 15 in a pharmaceutically acceptable carrier, diluent or adjuvant.

30

40. A method of treating an indication with a sustained-release composition according to Claims 1 or 15 in a pharmaceutically acceptable carrier, diluent or adjuvant.

35

41. A method of treatment of a disorder selected from the group consisting of excess weight, diabetes, high blood lipid level, arterial sclerosis,

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artherial plaque, the reduction or prevention of gall stones formation, insufficient lean tissue mass, insufficient sensitivity to insulin, and stroke, with a sustained-release composition according to Claims 1 or 5 15 in a pharmaceutically acceptable carrier, diluent, or adjuvant wherein the biologically active agent is leptin, an analog or derivative thereof

42. A method of treating a disorder selected 10 from the group consisting of hematopoietic cell deficiencies, infection, and neutropenia with a sustained-release composition according to Claims 1 or 15 in a pharmaceutically acceptable carrier, diluent, or adjuvant wherein the biologically active agent is G- 15 CSF, an analog or derivative thereof.

43. A method of treating inflammation with a sustained-release composition according to Claims 1 or 15 in a pharmaceutically acceptable carrier, diluent, 20 or adjuvant, wherein the biologically active agent is an IL-1ra, an analog or derivative thereof.

INTERNATIONAL SEARCH REPORT

Interr. Appl. Application No

PCT/US 98/07566

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 08551 A (DAMON BIOTECH) 9 August 1990 see claims 19,21,23 see page 8; example 2 ---	1-13, 18-21, 23-31, 37-40
X	EP 0 531 611 A (EURO-CELTIQUE) 17 March 1993 see claims 14,15,17,24 ---	1-10, 18-21, 23-28, 37-40
	-/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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21 August 1998

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Inter. Appl. No.

PCT/US 98/07566

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 90 11757 A (DEPOMED SYSTEMS) 18 October 1990</p> <p>see claim 1</p> <p>see examples 4,5</p> <p>-----</p>	<p>1-10, 18-21, 23-28, 37-40</p>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 07566

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 40-43
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/07566

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9008551	A	09-08-1990	AU 5107490 A CA 2046324 A EP 0457837 A JP 4502768 T	24-08-1990 27-07-1990 27-11-1991 21-05-1992

EP 531611	A	17-03-1993	US 5215758 A AT 145820 T AU 653048 B AU 2107392 A CA 2065210 A DE 69215612 D DE 69215612 T DK 531611 T EP 0698389 A ES 2097826 T GR 3022572 T IL 101758 A JP 5148159 A US 5508043 A	01-06-1993 15-12-1996 15-09-1994 18-03-1993 12-03-1993 16-01-1997 15-05-1997 26-05-1997 28-02-1996 16-04-1997 31-05-1997 05-12-1996 15-06-1993 16-04-1996

WO 9011757	A	18-10-1990	US 5007790 A AT 106717 T AU 638012 B AU 5523490 A CA 2014451 A,C DE 69009755 D DE 69009755 T EP 0467975 A JP 4507088 T	16-04-1991 15-06-1994 17-06-1993 05-11-1990 11-10-1990 14-07-1994 20-10-1994 29-01-1992 10-12-1992

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